



purview of the practicing artist, and thus no criticality is seen in the selected pH ranges. It is my belief that the methods of extraction used by Breithaupt are significantly less efficient than, and a such unsuitable for, the methods of the instant invention.

The Table enclosed herewith describes experimental results obtained by the present inventors illustrating the superior and unexpectedly efficient extraction of total, and highly deesterified carotenoids from whole homogenized red pepper tissue extracted by the mild alkaline extraction, compared to that of the same material extracted by the methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4) solvent of Breithaupt.

These results conclusively prove that methods for extraction and deesterification of carotenoids, comprising alkaline ethyl acetate extraction of the carotenoids, as described and claimed in the instant application do indeed provide unexpected and significantly superior results as compared to the prior art methods, thereby overcoming the rejections of claims 95, 96, 98, 100-101, 103, 109-111, 120 under 35 U.S.C. § 102(a) and 103(a).

***Ethyl acetate vs methanol/ethyl acetate/light petroleum (1:1:1) extraction of deesterified carotenoids:***


Following deesterification with lipase, the carotenoid fraction was extracted by ethyl acetate, under alkaline pH, and methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4). For alkaline ethyl acetate extraction, the lipase reaction mixture was made alkaline with NaOH, to pH 9.5 (with 0.1N NaOH solution), and ethyl acetate was then added at a ratio of 1 volume ethyl acetate to 5 volumes aqueous deesterification mixture, the phases mixed and allowed to separate. For methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4) extraction, the solvents were added to the lipase reaction mixtures at a ratio of 1 volume solvents to 5 volumes aqueous deesterification mixture, the phases mixed and allowed to separate.

The aqueous phase was removed, the solvent phase reextracted four times with distilled water (water:solvent = 4:1 volume /volume), followed by drying by addition of superfluous sodium sulfate. The remaining solvent was then evaporated under vacuum in a Rotovapor (Buchi), and the resulting extracted oleoresin analyzed by HPLC, as described in the instant specification.

Treatments	MEOH: petrol- ether: ETA (1:1:1)- (According to Breithaupt)	ETA (According to Kanner et al.)
Extraction efficiency (1:4, aqueous: solvent)	33%	90%
Extraction efficiency (1:2, aqueous: solvent)	21%	78%

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

March 28, 2006



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ARO, Volcani Center,  
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*Enc.:*

CV of Joseph Kanner

Joseph Kanner

September 2005

**CURRICULUM VITAE****I. Personal**

Born at 1940

Married + 1

**II. University Education and Additional Training**

1961-1963	B.Sc., Faculty of Agriculture, Hebrew University of Jerusalem.
1965-1967	M.Sc., Faculty of Agriculture, Hebrew University of Jerusalem.
1970-1974	Ph.D. Thesis submitted to the Hebrew University of Jerusalem.
1974-1975	Post-doctorate at the Department of Nutrition and Food Science, M.I.T., Cambridge, U.S.A.
1981-1982	Sabbatical as Visiting Professor at the Department of Food Science, Cornell University, Ithaca, U.S.A.
1991-1992	Sabbatical as Visiting Professor at the Department of Food Science and Technology, University of Cal. Davis, U.S.A.

**III. Positions Held and Academic Status**

1968-1976	Research Scientist in Food Chemistry and Technology.
1976-1979	Head of research unit in Chemistry and Biochemistry of Citrus Products.
1976- to date	Head, unit of Food Chemistry and Biochemistry.
1985-1992	Head, Food Science Department, ARO, Volcani Center.
1989-to date	Professor adjunct, Department of Food Science. The Hebrew University, Faculty of Agriculture, Rehovot.
1995-1998	Director of the Institute for Technology & Storage of Agricultural Products.

**IV. Teaching and Training Experience (including tutorship of students)**

1976-1978	Lecture of the course on Chemistry of Foods, at the Agricultural High School, Rupin
1979	Teaching the course on Chemistry and Biochemistry of Foods, at the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot.
1979	Teaching the course on Analysis and Food Chemistry, at the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot.
	To date, 21 graduate students of the Hebrew University have carried out their M. Sc. thesis under my supervision:

### **Professional and Academic Status**

1990-to date Researcher Scientific Grade A+ (equivalent to full Professor).

### **Functions in Professional Societies**

1978-1980 Chairman of the Israel Society for Food and Nutrition Sciences, affiliated to the I.F.T.

1993-to date Committee Member of the Israel Society for Oxygen and Free Radicals Research.

1999- President elected of the Israel Society for Oxygen and Free Radicals Research.

### **VIII. Membership in Professional Societies**

Member of the Israel Society for Food and Nutrition Science.

Member of the I.F.T. Society.

Member of the Society for Free Radical Research.

### **IX. Contribution to the Scientific Community**

During my career as a researcher, Department Head and Director of the Institute and as the Chairman of two societies, I organized many seminars and symposia in the area of food science and free radical research.

### **XI. Awards and honors.**

2001 ISI honors for "Highly Cited Researcher" (1981-2001) in recognition for outstanding achievements and contributions to the international research community and one of the most influential researcher in the field of Agriculture.

2002 The Israel Academy of Science found our papers in the field of Chemistry to be the most highly cited in Israel (between the first 200 papers) for the years (1981-2001).

### **XII. LIST OF PUBLICATION**

#### **A. Reviewed articles.**

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2.b Palevith, D., Harel, S., Kanner, J. and Ben-Gera, I. The effect of preharvest sweet dehydration on the composition of once-over harvest sweet paprika. Scientia Horticulture. 1975, 3: 143-148.

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- 4.a Kanner, J. and Harel, M. Changes in lysozyme due to reactions with peroxidizing methyl linoleate in dehydrated model system. *J. Agric. Food Chem.* 1976, 24: 486-472.
- 5.a Kanner, J., Mendel, H. and Busowski, P. Prooxidant and antioxidant effects of ascorbic acid and metal salts in a  $\beta$ -carotene-linoleate model system. *J. Food Science.* 1977, 42: 60-64.
- 6.a Kanner, J., Mendel, H. and Budowski, P. Carotene oxidizing factors in red pepper fruits (*Capsicum annum* L.): Peroxidase-like protein. *J. Food Sci.* 1977, 42: 1549-1551.
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- 8.b Juven, B.J., Kanner, J. and Weisslovitz, H. Influence of orange juice composition on the thermal resistance of spoilage yeasts. *J. Food Sci.* 1978, 43: 1074-1076.
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- 10.a Kanner, J., Mendel H. and Budowski, P. Carotene oxidizing factors on red pepper fruits (*Capsicum annum* L.): Oleoresin-cellulose solid model. *J. Food Sci.* 1978, 43: 709-712.
- 11.a Harel, S., Kanner, J., Juven, B.J. and Golan, R. Long-term preservation of high-moisture dried apricots with and without chemical preservatives. *Lebensm-Wiss. M-Technol.* 1978, 11: 219-221.
- 12.a Kanner, J., Elmaleh, H., Reuveni, O. and Ben-Gera, I. Invertase ( $\beta$ -fructofuranosidase) activity in three date cultivars. *J. Agric. Food Soc.* 1978, 27: 1238-1240.
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- 14.a Kanner, J., Harel, S. and Mendel, H. Content and stability of  $\alpha$ -tocopherol in fresh and dehydrated pepper fruits (*Capsicum annum* L.). *J. Agric. Food Chem.* 1979, 27: 1316-1318.
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- 16.a Kanner, J. Nitric-oxide myoglobin as an inhibitor of lipid oxidation. *Lipids*, 1980, 15: 944-948.
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